The Molecular Epidemiology of Clostridium difficile in Canadian Hospitals: 2005-2009

Mulvey, M.R., 1 Boyd, D.A., 1 Du T., 1 Hizon, R., 1 Miller, M., 1 Gravel, D., 1 McGeer, A., 1 Moore, D., 1 Simor, A., 1 Suh, K., 1 Taylor, G., 1 and the Canadian Nosocomial Infection Surveillance Program.


Abstract

Objective: The emergence of hypervirulent strains causing serious Clostridium difficile infections (CDI) is a growing concern in Canada and many other countries. This study examined the molecular epidemiology of C. difficile from 2005 to 2009 in Canada.

Methods: In April and May 2005, 2006, 2008, and 2009, all toxin positive isolates identified from inpatients at 29 sites from the Canadian Nosocomial Infection Surveillance Program (CNISP) were submitted to the National Microbiology Laboratory. C. difficile was isolated using an alcohol shock procedure. PCR was used to detect the toxin genes and tcdC gene, to confirm the species type, to detect mutations in the 5’ UTR, and to detect the presence of binary toxin (ctxC). Isolates were typed by PFGE using Swal and Fingerprinting were analyzed with BioNumerics (v. 5.10).

Results: A total of 1,225 C. difficile isolates were typed with 12 North American PulseNet (NP) types identified over the study period. The three most common strains identified were NAP1 (42.5%, n=521), NAP2 (31.5%, n=390), and NAP17 (9%, n=111). NAP1 increased from 28.6% (2005) to 48% (2008), and first slightly declined to 41% (2009), before increasing again to 48% (2010). Overall, the number of NAP strains increased from 13% in 2005 to 22% in 2010.

Conclusions: In general, C. difficile strains that have been reported to be more virulent than the NAP1 strains (e.g., NAP1+ isolates) are increasing in Canada and seem to be replacing other strains without ctxC deletions. However, the NAP strains with a wide range of strains have continued to increase in wide in Canada. Further studies into the virulence and fitness of these epidemic strains should be undertaken.

Materials & Methods

Surveillance Design: The locations of the hospital sites involved in this study are shown below. In April and May 2005, 2006, 2008, and 2009, all toxin positive isolates identified from the Canadian Nosocomial Infection Surveillance Program (CNISP) were submitted to the National Microbiology Laboratory (NML). Eighty isolates collected from the combined clinical and surveillance periods were wild-type for -20% and confirmed toxin positive by 16S RNA sequencing. A second set of 120 clinical and surveillance isolates sent to be on the NML for the study was confirmed toxin positive by 16S RNA sequencing and applied for the typing study.

Molecular Characterization: An automated PCR was used to confirm the species (cys) and detect ctx (e). A second multiplex was used to detect variations in the -20% gene, detect the ctxC and cdtA genes (4). Pull-down gel electrophoresis (PEG) was used to type the strains using the restriction enzyme SmaI. Fingerprinting analysis was performed using BioNumerics (v. 5.10).

Background

Clostridium difficile is a significant cause of morbidity and mortality in humans and is also the most common cause of nosocomial diarrhea (1). In this century, a hypervirulent strain of C. difficile, designated NAP1, has emerged in Canada and the United States (2). In Canada, this strain was first observed in Quebec, but then rapidly disseminated across Canada (2, 3). The NAP1 strain producing increased amounts of toxin C and has been associated with an increase in disease severity in certain age groups (5, 6). A new strain of C. difficile designated ribotype 078 (NAP9), has been reported in Europe and North America (7). The changing epidemiology of the Canadian Nosocomial Infection Surveillance Program (CNISP) has been monitoring C. difficile infections in Canada. In this report we describe the changing molecular epidemiology of C. difficile in Canada.

Conclusions

1. In 2005, 2006, 2008, and 2009, we compared 68% of all isolates versus approximately 20% of isolates not falling within the 12 NP types strains.

2. Over the study period, NAP6, NAP9, and non-NAP6 strains appear to have replaced the NAP1. In Canada.

3. There was an increase in the proportion of NAP6 in the central region (Ontario and Quebec) between 2008 (6%) and 2009 (17%).

4. Although the proportion of the hypervirulent NAP1 strain is low in Canada, NAP18 has increased from 1.2% in 2005 to 2.3% in 2010.

Results: Percentage of NAP Types for 2005-2009

References


Acknowledgments

We wish to thank the following institutions for their efforts in patient isolation, identification, and reporting accuracy: Members of the Minnesota Nosocomial Infection Surveillance Program; David Boyd, National Microbiology Laboratory, Public Health Agency of Canada; Elizabeth Knox, Vancouver General Hospital, Vancouver; John Conly, Forensic Medical Centre, Calgary, Alberta; Jane Dickens, Kirkcaldy General Hospital, Kirkcaldy, Scotland; Ainhoa Kereki, Health Care Centre, Menorca, Spain; Mark Sowden, Rocky Mountain Children’s Hospital, Edmonton, Alberta; Chris Freeman, McGill University Health Centre, Montreal, Quebec; Lauren Brennan, Health Unit Network, Toronto, Ontario; George Delaporte, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Manitoba; Centres for Communicable Disease and Infection Control, Public Health Agency of Canada, Elizabeth Hinchliffe, Peter McLachlan, Health Sciences Centre, St. John’s, Newfoundland; Michael Judd, London Health Sciences Centre, London, Ontario; Lynn Johansen, Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia; Keni Kane, North York General Hospital, Toronto, Ontario; Patrick MacNeil, Victoria General Hospital, Victoria, British Columbia; Bridget Kudo, South East Regional Health Authority, McMaster, Nova Scotia; Sonja Lamoureux, IWK Health Centre, Halifax, Nova Scotia; Mark Mullen, Shriners Children’s Hospital, Montreal, Quebec; Alejandro Membre, Centre for Communicable Diseases and Infection Control, Public Health Agency of Canada; Theresa Manley, Montreal Children’s Hospital, Montreal Children’s Hospital, Montreal, Quebec; Ahmed Marrakchi, Centre for Communicable Diseases and Infection Control, Public Health Agency of Canada; Michael Mulvey, National Microbiology Laboratory, Public Health Agency of Canada; Linda Rutledge, Centre for Communicable Diseases and Infection Control, Public Health Agency of Canada; Virginia Roth, The Ottawa Hospital, Ottawa, Ontario; Andrea Simon, Saskatchewan Health Sciences Centre, Regina, Saskatchewan; Kathy Sak, The Ottawa Hospital, Ottawa, Ontario; Jocelyn Coughlan, University of Alberta Hospital, Edmonton, Alberta; Eszter Thomas, Children’s and Women’s Health Centre, Vancouver, British Columbia; Nathan Vaught, Methok Diedo de los Muertos (DDH), Mexico; Mary Varney, Nonprofit Health Sciences Centre, Toronto, Ontario; Joseph Vajdyjad, Alberta Children’s Hospital, Calgary, Alberta; Karl Winter, McMaster University, Hamilton, Ontario; Kim Young, Royal Victoria Hospital, Vancouver, British Columbia; Kim Unckel, The Ottawa Hospital, Ottawa, Ontario; Joyce Giesbrecht, University of Alberta Hospital, Edmonton, Alberta; christine Gurney, King’s College Hospital, Kingston, Ontario.

Hospitals participating in CNISP CDAD Surveillance

Figure 1: Distribution of NAP Types by Study Year

Figure 2: Comparison of Regional Distribution of NAP Types

Figure 3: Fingerprinting of NAP Type Strains

Figure 4: Distribution of NAP Types by Study Year

Figure 5: Western Distribution of NAP Types by Study Year

Figure 6: Central Distribution of NAP Types by Study Year

Figure 7: Eastern Distribution of NAP Types by Study Year

Figure 8: Distribution of NAP Types in Canada

Figure 9: Distribution of NAP Types in Canada